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CENTRAL HISTAMINE NEURONS OF THE RAT SHOW AN ABNORMALLY LOW RESPONSE TO GLUTAMATE IN VITRO

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SUMMARY

1. Central histamine neurons appear to be unusually resistant to both hypoxia and to excitotoxic lesioning by glutamate receptor agonists.

2. Application of glutamate to hypothalamic histamine neurons *in vitro* produces an unusually small dose-dependent depolarizing response. An increased response of these neurons to glutamate in zero Mg^{+2} indicates that both NMDA and non-NMDA receptors may be present, but in significantly fewer numbers than in other neurons of the rat brain.

3. This arrangement may be due to a relative lack of synaptic afferents to these widely-projecting neurons. The destructive effects of excitatory amino acid release that usually accompanies hypoxia may therefore be absent in these neurons.

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WARNING

INTRODUCTION

The tuberomammilary (TM) nucleus of the hypothalamus contains the sole source of known histaminergic neurons in rat brain (Panula *et al.*, 1984; Watenabe *et al.*, 1985; Pollard *et al.*, 1985). This nucleus also appears to be unusually resistant to hypoxia (Reiner, Note 1) and to excitotoxic lesioning by the glutamate-receptor agonist ibotenate (_____, Note 2). It has been suggested that one major mechanism of cell death during anoxia is an increased Ca^{+2} influx due to glutamate-receptor activation by a massive release of excitatory amino acids. This mechanism is the same as that for excitotoxic cell death (Rothman and Olney, 1986), requiring in particular the NMDA type of glutamate receptor and its associated Ca^{+2} current (for a review see Choi, 1988). We hypothesized that histaminergic TM neurons may have significantly fewer glutamate receptors in general, or NMDA receptors in particular, compared to all other areas of the brain, and are thus relatively hypoxia tolerant. In the following pharmacological experiments the responsiveness of these neurons to glutamate was tested *in vitro*.

MATERIALS AND METHODS

Intracellular recordings were obtained from neurons of the TM nucleus in hypothalamic slices maintained *in vitro* using standard electrophysiological techniques. Young adult male rats (75-150 g) were anesthetized with ether, decapitated, and the brain was rapidly removed and immersed in ice-cold artificial cerebrospinal fluid (ACSF) of the following composition (in mM): Na^{+}, 152; K^{+}, 2.5; Ca^{+2}, 2.4; Cl^{-}, 136; PO_4^{-2}, 1.2; Mg^{+2}, 1.8; CO_3^{-}, 25; glucose, 11; pH 7.4 when saturated with 95% O_2 and 5% CO_2. The hypothalamus was dissected free and coronal slices of 400 μm thickness were prepared using a vibratome. Slices were stored in oxygenated ACSF at room temperature prior to being transferer singly to a recording chamber where they were

submerged and continually superfused with warmed (37°C), oxygenated ACSF flowing at 1-1.5 ml/min. Glutamate was applied at concentrations of 10, 25, and 50 μM for 1-2 minute periods directly to the perfusate, followed by a wash of at least 10 min. The Mg^{++} concentration of the perfusate was also varied, from normal (1.8 μM) to zero. The effect of glutamate on the resting membrane potential and apparent input resistance of three TM neurons was measured by application of depolarizing and hyperpolarizing current pulses (Fig. 1)

TM neurons were recorded from the readily identifiable ventral subnucleus as defined by Staines *et al* (1987), at the extreme ventral edge of the mammillary body at the level of the mammillary recess, using glass micropipettes drawn from 2.0 mm microfilament glass and filled with 3 M KCl. Following penetration, a hyperpolarizing current of 0.5 - 1.0 nA was applied for several minutes. Subsequently, hyperpolarizing current sufficient to maintain the membrane just below threshold was applied, as TM neurons are usually spontaneously active. Stable intracellular recordings lasted 2-4 hours. Records were obtained from a digital oscilloscope and a chart recorder.

The three neurons studied exhibited a constellation of membrane properties previously described by Haas and Reiner (1988) for positively identified histaminergic TM neurons: spontaneous activity; half-amplitude spike duration at the resting potential of 1-2 msec with a notch on the descending limb; long afterhyperpolarizations of 20 mV and at least 220 msec duration; apparent input impedance of 225 megohms; a charging time constant of 25 msec; and a regenerative calcium conductance contributing to the action potential, as revealed by the addition of tetrodotoxin and tetraethylammonium ion. The characteristic inward and transient outward rectification of TM neurons was not conclusively demonstrated, as addressed in the Discussion section.

RESULTS

Bath application of glutamate resulted in small dose-dependent depolarizations and decreases of apparent input impedence of the recorded TM neurons. Table 1 displays the values for peak depolarizations. This depolarization response was roughly doubled by the removal of Mg^{+2} from the perfusate. An example is shown in Figure 2.

INSERT TABLE 1 & FIGURE 2 ABOUT HERE

Table 1 shows that some TM neurons (cells one and two) may be more responsive than others (cell three) to glutamate both in normal and zero Mg concentrations. The more responsive neuron (cell one) also showed significant fluctuations in resting potential upon switching to a zero Mg bath (20 mV over 5 min prior to stabilizing).

DISCUSSION

The voltage-dependent Mg^{+2} block of the channel or of the subconductance state associated with the NMDA-type receptor is removed when the extracellular Mg^{+2} is removed (Nowak *et al*, 1984; see also Jahr & Stevens, 1987). The increased response by these TM neurons to glutamate in zero Mg^{+2} therefore indicates that histaminergic neurons may express both NMDA and non-NMDA receptors. The peak response of these neurons to glutamate, however, is considerably smaller than responses observed from neurons of other rat brain sites. Ten µM glutamate is sufficient for patch clamp recording of every subconductance state of glutamate receptor-coupled channels in hippocampal cultures (Jahr & Stevens, 1988), as well as in cerebellar Purkinje cell cultures (Cull-Candy & Usowicz, 1988). Ten µM glutamate added to the perfusate in 1.8 mM Mg^{+2} is also enough to produce 20% cell death after five minutes in hippcampal

cultures, with 90% cell death in zero Mg^{+2}(Finkenbeiner & Stevens, 1988). In cortical neuron cultures, 50 μM glutamate will kill half the cells in five minutes (Koh & Choi, 1988).

The possibility that these TM neurons are not histaminergic or are otherwise atypical has been considered. The presence of either inward rectification or transient outward rectification conclusively distinguishes histaminergic TM neurons from the rare atypical TM neurons (Haas and Reiner, 1988), which have not been conclusively identified as histaminergic. The invariably-present transient outward rectification of histaminergic TM neurons during recharging can be demonstrated by a semi-logrithmic plot of charging and discharging membrane potentials, in which the discharging does not conform to a single exponential process (Haas and Reiner, 1988). In this study the resolution of current-clamp recordings was not great enough to reliably plot the tail of the discharging component, but as shown in Figure 1 the discharging component does appear to approach a different asymptote. The I-V plot generated from 100 ms pulses of hyperpolarizing current from a holding membrane potential just below threshold was consistent with the described inward rectification for TM neurons (Haas and Reiner, 1988), but the range of hyperpolarizing pulses was not sufficient to make a reliable comparison, and the holding potential was not varied to show the characteristic voltage dependence of this inwardly rectifying current. Although these two rectifying currents were not conclusively demonstrated for the recorded TM neurons, the atypical TM neurons are quite rare and differ markedly from the normal profile in other ways, including action potential durations of less than 1 ms and after-hyperpolarizations of less than 20 ms (Haas and Reiner, 1988). Therefore the neurons in this study have been designated as being typical histaminergic neurons.

Known afferents to TM neurons are GABAergic ("reference", 1988). In this study the equilibrium potential of chloride ions was shifted so that IPSPs became EPSPs, and thus inhibitory influences from GABA-A receptors can be ruled out as being responsible for the weak response to glutamate. This procedure, however, does call into question the possibility that the cells from which recordings were made were not being excited by glutamate directly, but by way of other neurons (or severed axons) with normally inhibitory inputs to the recorded cells. Such inhibitory inputs are likely to be from other glutamate-responsive histaminergic neurons, since (a) afferents to the TM nucleus appear to be very rare, (b) TM neurons are glutamic acid decarboxylase-immunoreactive (Kohler *et al.*, 1985), and (c) TM neurons appear to have extensive dendro-dendritic contact (Reiner *et al.*, 1987). In this case the response to glutamate of the primary neurons could be larger than the response of the recorded neurons. Two further possible confounds are (a) that the glutamate application stimulated the reversed chloride current by direct potentiation of GABA receptors (Stelzer and Wong, 1989), or (b) that cyclic-nucleotide mediated modulation may have arisen from direct histamine re-afferents or colaterals (Wamsley and Palacios, 1984). In no instance, however, was an increase in spontaneous EPSPs observed, so the above possible confounds are unlikely.

TM neurons may express low numbers of both NMDA and non-NMDA receptors. The conclusion that the small responses to glutamate in normal Mg^{+2} were due to non-NMDA receptors is warranted by the fact that evoked responses of hippocampal NMDA receptors are inhibited by as small as 30 uM Mg^{+2} (Stevens and Finkbeinder, 1988), so that depolarization of around 30 mV would have been necessary to activate these NMDA receptors. Coactivity of NMDA and non-NMDA channels may be required for *in-vivo* excitotoxic damage (Choi, 1988). The small depolarizations of TM neurons in response to

glutamate in normal Mg indicates an arrangement in which the non-NMDA receptor-gated Na^{+} currents cannot depolarize the membrane above the NMDA-receptor voltage threshold. In the case that the NMDA receptors do become activated (such as by the removal of Mg^{+2} in this study), their collective Ca-current response remains minimal.

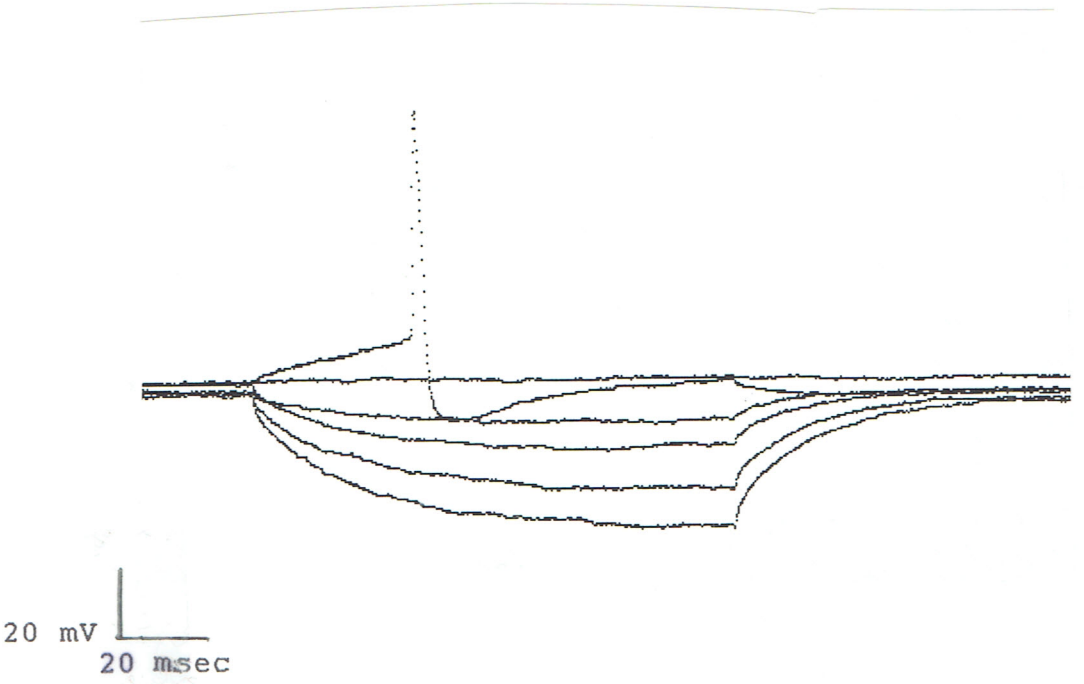
Central histamine neurons may be unusual in that they may display a relative lack of glutaminergic afferents. The present data is consistent with the hypothesis that most input to the TM nucleus is humoral rather than synaptic, by way of these neurons' extensive dendritic interfaces with the ependyma of the mammillary recess and ventral hypothalamus (Staines *et al.*, 1987; Reiner *et al.*, 1987). GABAergic inhibition of these neurons may be largely due to dendritic release from cells intrinsic to the TM nucleus, including the histamine neurons themselves. Reduced glutamate sensitivity may be a consequence of this arrangement.

Expression of receptors on the cell membrane without physiological function is unlikely for a molecule as structurally and functionally elaborate as the NMDA receptor-channel complex (MacLennan, Note 3). If glutamate-receptor excitation of TM neurons *in situ* is largely from humoral sources, then the increased synaptic activity that produces local toxic glutamate levels in hypoxic states (Rothman, 1983) may not occur for these neurons. The histamine neurons of the TM nucleus may therefore be an excellent site for non-intrusive study of the *in-situ* effects of anoxia independent of excitotoxic influences.

FIGURE LEGENDS

FIGURE 1. A family of depolarizing and hyperpolarizing current pulses at .05, 0, -.05, -.10, -.15, and -.20 nA for 100 msec were applied repetitively to each TM neuron prior to and during test applications of glutamate.

FIGURE 2. A. Chart record demonstrating the depolarization of the resting potential and the decrease in apparent input impedence of the membrane of cell three in response to application of glutamate for one minute, as measured by voltage responses to current pulses like those of Figure 1. The small effect of 25 μM glutamate in normal Mg^{+2}_{o} is shown in A. B. The same conditions as in A but with zero Mg^{+2} in the perfusate, producing a significantly larger depolarization and decrease in apparent input impedence.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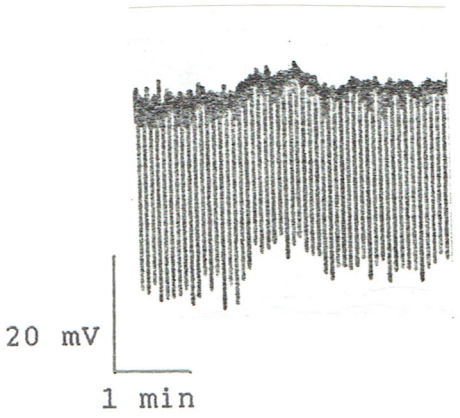

A.

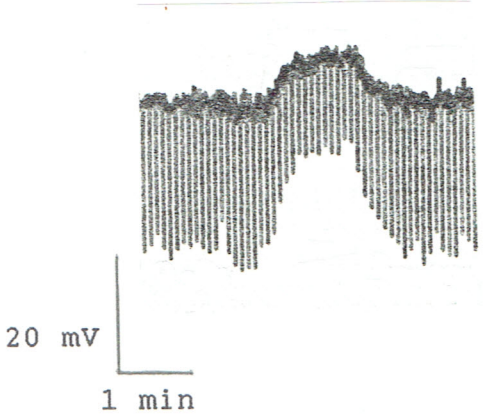

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TABLE LEGENDS

Table 1. Peak depolarization from baseline (in mV) in response to glutamate application for two min in three TM neurons in normal and zero Mg^{+2} concentrations in the perfusate. In each condition, glutamate was applied at concentrations of 10, 25, and 50 μM. Dose dependent responses are seen, and are potentiated by reduced Mg^{+2}.

	Normal Mg (1.8 uM)			Zero Mg		
Glutamate	10 uM	25 uM	50 uM	10 uM	25 uM	50 uM
Cell 1	12 mV			20 mV		
Cell 2	12 mV					
Cell 3	3 mV	4 mV	7 mV	5 mV	10 mV	20 mV

REFERENCES

CHOI, D.W. (1988). Calcium-mediated neurotoxicity: relationship to specific channel types and roles in ischemic damage. *Trends in Neuroscience* 11, 465-469.

CULL-CANDY, S.G., & USOWICZ, M.M. Multiple-conductance channels activated by excitatory amino acids in cerebellar neurons. *Nature* 325, 525-528.

HAAS, H.L. & REINER, P.B. (1988). Membrane properties of histaminergic tuberomammilary neurones of the rat hypothalamus *in vitro*. *Journal of Physiology* 399, 633-646.

FINKBEINER, S. & STEVENS, C.F. (1988). Applications of quantitative measurements for assessing glutamate neurotoxicity. *Proceedings of the National Academy of Science, USA* 85, 4071-4074.

JAHR, C.E. & STEVENS, C.F. (1987). Glutamate activates multiple single-channel conductances in hippocampal neurons. *Nature* 325, 522-525.

KOH, J.Y., & CHOI, D.W. (1988). Vulnerability of cultured cortical neurons to damage by excitotoxins: differential susceptibility of neurons containing NADPH-diaphorase. *Journal of Neuroscience* 8, 2153-2163.

KOHLER, C., SWANSON, L.W., HAGLUND, L. & WU, J-L. (1985). The cytoarchitecture, histochemistry and projections of the tuberomammilary nucleus on the rat. *Neuroscience* 16, 85-110.

NOWAK, L., BREGESTOVSKI, P., ASCHER, P., HERBERT, A. & PROCHIANITZ, A. (1984). Magnesium gates glutamate-activated channels in mouse central neurones. *Nature* 307, 462-465.

PANULA, P. YANG, H-Y. T., & COSTA, E. (1984). Histamine-containing neurons in the rat hypothalamus. *Proceedings of the National Academy of Science, USA* 81, 2572-2576.

POLLARD, H., PACHOT, I., & SCHWARTZ, J-C. (1985). Monoclonal antibody against L-histidine decarboxylase for localization of histaminergic cells. *Neuroscience Letters* 54, 53-58.

REINER, B.P., SEMBA, K., WATANABE, T. & WADA, H. (1987). En bloc immunohistochemistry reveals extensive distribution of histidine decarboxylase immunoreactive neurons on the ventral surface of the rat hypothalamus. *Neuroscience Letters* 77, 137-142.

ROTHMAN, S.M. (1983). Synaptic activity mediates death of hypoxic neurons. *Science* 220, 536-537.

ROTHMAN, S.M. & OLNEY, J.W. (1985). Glutamate and the Pathophysiology of hypoxic-ischemic brain damage. *Annals of Neurology* 19, 105-111.

STAINES, W.A., DADDONA, P.E. & NAGY, G.I. (1987). The organization and hypothalamic projections of the tuberomammilary nucleus in the rat: an immuno-histochemical study of adenosine deaminase-positive neurons and fibers. *Neuroscience* 23, 571-596.

STELZER, A. & WONG, R.K.M. (1989). Gaba-A responses in hippocampal neurons are potentiated by glutamate. *Nature* 337, 170-174.

WAMSLEY, J.K. & PALACIOS, J.M. (1984). Histaminergic receptors. In *Handbook of Chemical Neuroanatomy*, Vol. 3: *Classical Transmitters and Transmitter Receptors in the CNS*, Part II, Ed.: BJORKLAND, A, HOKFELT, T., & KAHUR, M., pp 386-406.

WATANABE, T., TAGUCHI, Y., SHIOSAKA, S., TANAKA, J., KUBOTA, H., TERANO, Y., TOHYAMA, M., & WADA, H. (1984). Distribution of the histaminergic neuron system in the central nervous system of rats; a fluorescent immuno-histochemical analysis with histidine decarboxylase as a marker. *Brain Research* 295, 13-25.

REFERENCE NOTES

Note 1. REINER, P.B., personal observation, University of British Columbia, Department of Psychiatry, 1988.

Note 2. _____, personal communication, _____, 1989.

Note 3. McLENNAN, H., personal communication, University of British Columbia, Department of Physiology, 1989.